Sample preparation: Condition a 6 mL Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Filter 30 mL microsomal incubation, add filtrate to SPE cartridge, elute with MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm C18 (Alltech)
Mobile phase: Gradient. MeCN:200 mM pH 6.5 ammonium acetate 10:90 for 5 min, to 20:80 over 5 min, maintain at 20:80 for 10 min, to 50:50 over 10 min.
Flow rate: 1
Detector: UV 254, UV 362

CHROMATOGRAM

Retention time: 35

OTHER SUBSTANCES

Extracted: metabolites

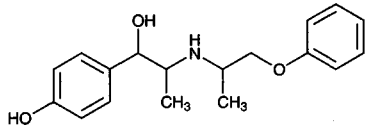
KEY WORDS

rabbit; liver; SPE

REFERENCE

Woolf,T.F.; Black,A.; Chang,T. In vitro metabolism of isoxicam by horseradish peroxidase, *Xenobiotica*, **1989**, *19*, 1369–1377.

Isoxsuprine



Molecular formula: $C_{18}H_{23}NO_3$
Molecular weight: 301.39
CAS Registry No.: 395-28-8, 579-56-6 (HCl)
Merck Index: 5259
Lednicer No.: 1 69

SAMPLE

Matrix: blood
Sample preparation: Dilute blood with an equal volume of water. 1 mL Plasma or 900 μL diluted blood + 0.9-1 mL buffer + 5 mL freshly distilled ethyl acetate, vortex for 1 min, centrifuge at 1750 g for 7 min. Remove the organic layer and evaporate it almost to dryness under a stream of nitrogen at 57°, evaporate the final 500 μL at room temperature, reconstitute the residue in 100 μL MeCN, vortex for 15 s, inject the whole amount. (Buffer was 26.5 g sodium carbonate and 21 g sodium bicarbonate in 500 mL water, pH 9.48.)

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak phenyl (plasma) or 200 × 4.6 5 μm Spheri-5 RP-18 (blood)
Mobile phase: MeCN:0.05% orthophosphoric acid 17:83 (plasma) or 63:37 (blood)
Flow rate: 2
Injection volume: 100
Detector: F ex 200 no emission filter or UV 254

CHROMATOGRAM

Retention time: 15.1 (plasma), 16.3 (blood)
Internal standard: isoxsuprine hydrochloride

OTHER SUBSTANCES

Extracted: ritodrine
Simultaneous: fenoterol

Noninterfering: acetaminophen, albuterol, betamethasone, bupivacaine, caffeine, chloral hydrate, dexamethasone, diazepam, lignocaine, meperidine, metoclopramide, morphine, nitrazepam, terbutaline

KEY WORDS
isoxsuprine is IS; plasma; whole blood

REFERENCE
Gross,A.S.; Brown,K.F.; Baird-Lambert,J.A.; Nation,R.L. Determination of ritodrine in blood and plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1987**, *416*, 400–408.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + nylidrin + 500 μL buffer, mix briefly, add 50-75 mg resin, mix at 10-25 rpm for 30 min, discard the supernatant, wash twice with 1 mL buffer, add 500 μL 50 mg/mL KOH in MeOH:water 50:50, mix for 30 min, inject a 20 μL aliquot of the eluate. (Buffer was 100 mM citric acid:200 mM Na_2HPO_4 29:71, pH 6.5 (McIlvaine buffer) Wash 20-50 mesh Dowex HCR-S resin twice with water and allow it to equilibrate in buffer).

HPLC VARIABLES
Guard column: 25 × 4.6 5 μm Spherisorb ODS-I
Column: 250 × 4.6 5 μm Spherisorb ODS-I
Mobile phase: MeCN:MeOH:buffer 30:18:52 containing 1.8 mM octanesulfonic acid (Buffer was 30 mM KH_2PO_4 adjusted to pH 3.0 with concentrated orthophosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: E, Gynotek M20, glassy carbon working electrode 950 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 8.5
Internal standard: nylidrin (11)
Limit of detection: 1 ng/mL (from 2 mL plasma)
Limit of quantitation: 5 ng/mL

KEY WORDS
horse; plasma; pharmacokinetics; SPE

REFERENCE
Hashem,A.; Lubczyk,B. Determination of isoxsuprine in equine plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1991**, *563*, 216–223.

SAMPLE
Matrix: blood
Sample preparation: Condition a Baker 500 mg C18 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Serum + 714 units β-glucuronidase (E. coli, Sigma), heat at 37° overnight, add 2 mL water, add to the SPE cartridge at 0.15 mL/s, wash with 3 mL water, elute with 2 mL MeOH:triethylamine 99:1. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm LC 8 DB (Supelco)
Mobile phase: Gradient. MeOH:buffer 5:95 for 1 min, to 95:5 over 20 min, maintain at 95:5 for 5 min, re-equilibrate at initial conditions for 10 min. (Buffer was 100 mM sodium acetate containing 0.1% triethylamine adjusted to pH 3.4 with 85% phosphoric acid.)
Flow rate: 1.2
Injection volume: 20
Detector: UV 276

CHROMATOGRAM
Limit of detection: 400 ng/mL

KEY WORDS
serum; horse; SPE; pharmacokinetics

REFERENCE
Pompa,G.; Caloni,F.; Montana,M.; Pasqualucci,C. Prolonged presence of isoxsuprine in equine serum after oral administration, *Xenobiotica*, **1994**, *24*, 339–346.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

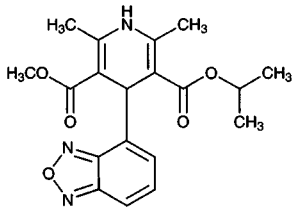
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3014 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 276

KEY WORDS
chiral; α = 1.40 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

Isradipine



Molecular formula: $C_{19}H_{21}N_3O_5$
Molecular weight: 371.39
CAS Registry No.: 75695-93-1
Merck Index: 5260
Lednicer No.: 4 107

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 4 ng/mL IS in water + 100 μL 2 M NaOH + 6 mL dichloromethane, mix on a rotary mixer for 20 min, centrifuge at 1500 g for 5 min (if emulsions form stir with a class rod and re-centrifuge). Remove the organic layer and evaporate it under a stream of nitrogen at 40°, add 500 μL dichloromethane, vortex, evaporate under a stream of nitrogen at 40°, reconstitute in 100 μL mobile phase, vortex for 30 s, allow to stand for 10 min, vortex, inject a 75 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-pak C18
Mobile phase: MeOH:10 mM dibutylamine phosphate (Waters D-4) 50:50, pH 2.8-3.0
Column temperature: 48
Flow rate: 1
Injection volume: 75
Detector: UV 325

CHROMATOGRAM
Retention time: 12
Internal standard: PY 108-068 (diethyl ester of isradipine) (13)
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma

REFERENCE

Boutagy,J.; Rumble,F.; Dunagan,F. Determination of isradipine and the oxidative pyridine metabolite in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *487*, 483–488.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 22.352

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: cells, culture medium

Sample preparation: Cells. To 100 μL containing 40 × 10^6 cell suspension add 100 μL of a methanolic solution of p-phenoxyphenol and benzoylated spermine so as to give concentrations of 10.63 μm and 2815 nM, respectively. Vortex for 1 min, sonicate for 3 min, add 200 μL MeOH, vortex for 1 min, centrifuge at 2000 g for 5 min, inject a 50 μL aliquot of the supernatant. Culture medium. To 5 mL culture medium add 200 μL of a methanolic solution of p-phenoxyphenol and benzoylated spermine so as to give concentrations of 10.63 μm and 2815 nM, respectively. Adjust the pH to 8.0 with 1 M NaOH, add 5 mL chloroform (Caution! Chloroform is a carcinogen!), mix at 30 rpm for 30 min, centrifuge at 1000 g for 5 min, remove the chloroform layer. Adjust the aqueous phase to pH 3.0 with 1 M HCl, add 1 mL 100 mM pH 3 citrate buffer and 1 mL 37 mM tetrabutylammonium hydrogen sulfate. Extract the aqueous phase with 5 mL chloroform at 30 rpm for 30 min, centrifuge at 1000 g for 5 min. Combine the chloroform layers and evaporate them to dryness under a stream of nitrogen, dissolve the residue in 300 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Chromspher C8 (Chrompack)

Mobile phase: MeOH:THF:buffer 43.6:1:55.4 (Buffer was water containing 160 mM NaH_2PO_4 and 17 mM tetrabutylammonium hydrogen sulfate, pH 3.0.)

Flow rate: 1

Injection volume: 50

Detector: UV 240

CHROMATOGRAM
Retention time: 37
Internal standard: p-phenoxyphenol (19), benzoylated spermine (33)
Limit of detection: 110 pmol/10^6 (cells), 210 nM (culture medium)
Limit of quantitation: 350 pmol/10^6 (cells), 700 nM (culture medium)

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Bidouil,S.; Dubois,J.; Hanocq,M. Isocratic high-performance liquid chromatographic method for the separation of isradipine and its main metabolites. Application to in vitro metabolization by h3A4/OR cells, *J.Chromatogr.B*, **1997**, *693*, 359–366.

SAMPLE
Matrix: formulations
Sample preparation: Shake bottle by hand, dilute a 1 mL aliquot with MeOH:95% EtOH 1:1 to an expected isradipine concentration of 100 μg/mL, filter (0.22 μm), inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spheri-5 ODS (Applied Biosystems)
Mobile phase: MeOH:THF:water 42:20:38
Flow rate: 1
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
suspensions; stability-indicating

REFERENCE
MacDonald,J.L.; Johnson,C.E.; Jacobson,P. Stability of isradipine in an extemporaneously compounded oral liquid, *Am.J.Hosp.Pharm.*, **1994**, *51*, 2409–2411.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 100 μM solution in buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 column containing riboflavin binding proteins (Prepare as follows. Add riboflavin to saturate protein of egg yolk, homogenize with 3 volumes buffer, centrifuge, add the supernatant to a 500 × 30 column of DEAE-cellulose (Whatman) equilibrated with buffer, wash extensively with buffer to remove bound protein, elute riboflavin binding proteins (RFBP) with buffer containing 200 mM NaCl (RFBP has intense yellow color, absorption at 455 nm). Purify RFBP on a Sephadex G-100 column with 50 mM pH 7.5 Tris-HCl buffer as eluent, remove the bound riboflavin by extensive dialysis at pH 3.0. Add 4.5 g N,N-disuccinylimidyl carbonate to 3 g Nucleosil 5NH2 slurried in MeCN, filter, wash with MeCN, wash with 50 mM pH 7.5 phosphate buffer. Suspend 300 mg RFBP in 50 mM phosphate buffer, add the activated silica, mix gently for 2 h using a rotary evaporator, filter, wash with sterile water, wash with isopropanol:water 1:2, pack in a 100 × 4.6 column.) (Buffer was 100 mM pH 5.3 sodium acetate.)
Mobile phase: 50 mM pH 5.5 KH_2PO_4
Flow rate: 0.8
Injection volume: 20
Detector: UV

CHROMATOGRAM
Retention time: k' 8.57

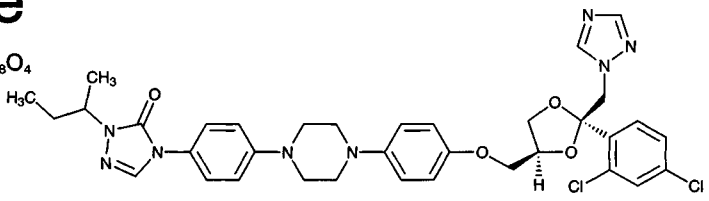
OTHER SUBSTANCES
Simultaneous: flurbiprofen, ketoprofen, nimodipine, suprofen

KEY WORDS
chiral; α = 1.28

REFERENCE
Massolini,G.; De Lorenzi,E.; Ponci,M.C.; Gandini,C.; Caccialanza,G.; Monaco,H.L. Egg yolk riboflavin binding protein as a new chiral stationary phase in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *704*, 55–65.

Itraconazole

Molecular formula: $C_{35}H_{38}Cl_2N_8O_4$
Molecular weight: 705.64
CAS Registry No.: 84625-61-6
Merck Index: 5262



SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 1 mg/mL IS in MeCN, add 500 mg KCl, vortex, centrifuge at 1500 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher RP8
Mobile phase: MeCN:water 55:45
Flow rate: 1.5
Injection volume: 40
Detector: UV 263

CHROMATOGRAM
Retention time: 10-11
Internal standard: R51012 (14-15) (Janssen, France)
Limit of detection: 20 μg/mL
Limit of quantitation: 40 μg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Cociglio,M.; Hillaire-Buys,D.; Alric,R. Prevalidation statistical design to assess analytical methods. Example of a quick liquid chromatographic assay of itraconazole in serum, *J.Chromatogr.B*, **1997**, *698*, 225–233.

SAMPLE
Matrix: blood
Sample preparation: 250 μL serum + IS + 50 μL 0.3 N barium hydroxide + 50 μL 0.4 N zinc sulfate + 1 mL MeCN, vortex, centrifuge at 3521 g for 15 min, evaporate the supernatant to dryness under a stream of nitrogen, reconstitute with 250 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 7.5 × 4.6 5 μm Alltech Alltima C18
Column: 250 × 4.6 5 μm Alltech Alltima C18
Mobile phase: MeCN:MeOH:50 mM pH 6.7 phosphate buffer 47:8:45
Column temperature: 37
Flow rate: 1
Detector: UV 263

CHROMATOGRAM
Internal standard: saperconazole
Limit of detection: 10 ng/mL
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; pharmacokinetics

REFERENCE
Christensen,K.J.; Gubbins,P.O.; Gurley,B.J.; Bowman,J.L.; Buice,R.G. Relative bioavailability of itraconazole from an extemporaneously prepared suspension and from the marketed capsules, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 261–265.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut SPE cartridge (No. 607101) with 1 mL MeOH and 1 mL water. 1 mL Serum + 100 μL 1 μg/mL IS in MeOH, mix, add to the SPE cartridge, wash with 1-2 mL water, wash with 1-2 mL MeOH:water 50:50, elute with 400 μL MeOH:triethylamine:concentrated orthophosphoric acid 99.7:0.3:0.3, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: 100 × 4.6 MPLC RP-18 Spheri-5
Mobile phase: MeCN:water 60:40 containing 20 mM triethylamine, pH adjusted to 2.3 with phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: F ex 260 em 365

CHROMATOGRAM
Retention time: 3
Internal standard: cis-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-5-methyl-2-(3-methylbutyl)-3H-1,2,4-triazol-3-one (R 51 012) (4)
Limit of quantitation: 4 ng/mL

OTHER SUBSTANCES
Noninterfering: amphotericin B, cefaclor, imipenem, flucytosine, gentamycin, ketoconazole, netilmicin, salicylic acid, sulfamethoxazole, tienamycin, tobramycin, trimethoprim

KEY WORDS
serum; SPE

REFERENCE
Allenmark,S.; Edebo,A.; Lindgren,K. Determination of itraconazole in serum with high-performance liquid chromatography and fluorescence detection [letter], *J.Chromatogr.*, **1990**, *532*, 203–206.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma or serum + 300 μL 250 nM IS in MeOH, vortex for 1 min, centrifuge at 2000 g for 2 min, inject a 250 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 50 × 4.6 20 μm Ultrasphere ODS
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:water:diethylamine 60:40:0.05 (At the end of each day flush column with MeOH:DMSO 90:10.)
Flow rate: 1.5
Injection volume: 250
Detector: UV 261

CHROMATOGRAM

Retention time: 6.1
Internal standard: cis-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-5-methyl-2-(3-methylbutyl)-3H-1,2,4-triazol-3-one (R51012) (8.5)
Limit of detection: 8 nM

OTHER SUBSTANCES

Noninterfering: amoxicillin, amphotericin B, ampicillin, cimetidine, diazepam, erythromycin, gentamycin, griseofulvin, ketoconazole, miconazole, nystatin, penicillin G, prednisolone, sulfamethoxazole, trimethoprim, zidovudine

KEY WORDS

plasma; serum

REFERENCE

Badcock,N.R. Micro-scale method for itraconazole in plasma by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *525*, 478–483.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 1 mL 50 mM sodium borate + 100 μL 10 μg/mL IS in MeOH + 200 μL MeOH, extract twice with 4 mL aliquots of heptane:isoamyl alcohol 95:5 in a rotary mixer. Combine the organic phases and add them to 2 mL 1 M sulfuric acid, extract. Discard the organic phase and add 600 μL concentrated ammonium hydroxide to the aqueous phase. Extract the aqueous phase twice with 2.5 mL portions of heptane:isoamyl alcohol 95:5. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL MeCN:water 60:40, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: C18
Column: 100 × 4.5 3 μm Hypersil octadecylsilane
Mobile phase: MeCN:water 40:60 containing 0.03% diethylamine adjusted to pH 7.8 with dilute orthophosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: k' 7.4
Internal standard: cis-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-5-methyl-2-(3-methylbutyl)-3H-1,2,4-triazol-3-one (R51012) (k' 10.8)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, hydroxyitraconazole

KEY WORDS

serum

REFERENCE
Law,D.; Moore,C.B.; Denning,D.W. Bioassay for serum itraconazole concentrations using hydroxyitraconazole standards, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1561–1566.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 1.5 mL MeOH, shake for 5 min, centrifuge. Remove the supernatant and evaporate it to dryness, reconstitute the residue in 100 μL MeOH, inject a 20 μL aliquot. Alternatively, condition a 100 mg Bakerbond C-18 SPE cartridge with 2 mL MeOH and 2 mL water. 1 mL Plasma + 1 mL water, add to the SPE cartridge, wash with 2 mL water containing 100 μL MeCN, elute with 2 mL MeOH or MeCN. Evaporate the eluate to dryness, reconstitute the residue in 100 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3 5 μm Separon SGX C-18 (Tessek, Prague)
Mobile phase: MeOH:water:triethylamine 72:28:0.05
Flow rate: 1.1
Injection volume: 20
Detector: UV (wavelength not specified)

CHROMATOGRAM
Retention time: 9
Internal standard: R 51012 (13)
Limit of detection: 10 ng/mL (?)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE; plasma; serum

REFERENCE
Brandsteterova,E.; Kubalec,P.; Rády,A.; Krcméry,V. Determination of itraconazole and its metabolites in plasma using SPE-HPLC, *Pharmazie*, **1995**, *50*, 597–599.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 2 mL 95% EtOH and 2 mL MeCN:water 15:85. 200 μL Plasma or whole blood + 50 μL 100 μM testosterone propionate in MeOH + 3 mL MeCN:water 15:85, vortex for 30 s, add to the SPE cartridge, wash with 9 mL MeCN:water 30:70, dry, elute with 200 μL 95% EtOH, inject a 10 μL aliquot of the eluate.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil LC-8DB
Mobile phase: MeOH:buffer 72.5:27.5 (Buffer was 25 mM K_2HPO_4 adjusted to pH 3 with 670 mM phosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 2.55
Internal standard: testosterone propionate (3.60)

OTHER SUBSTANCES
Extracted: doxepin
Noninterfering: acetaminophen, N-acetylprocainamide, amitriptyline, aspirin, barbituric acid, brompheniramine, caffeine, carbamazepine, chloramphenicol, chlorpheniramine, clonazepam, desipramine, desmethyldoxepin, digitoxin, digoxin, disopyramide, ethosuximide, felbamate, gentamicin, ibuprofen, imipramine, lidocaine, maprotiline, mephenytoin, mephobarbital, meth-

arbital, methsuximide, methylsuccinimide, nortriptyline, paramethadione, phenacemide, phenobarbital, phensuximide, phenylpropanolamine, phenytoin, primidone, procainamide, protriptyline, quinidine, theophylline, tobramycin, trimethadione, valproic acid, vancomycin
Interfering: clotrimazole

KEY WORDS
plasma; SPE; whole blood

REFERENCE
Rifai,N.; Sakamoto,M.; Law,T.; Platt,O.; Mikati,M.; Armsby,C.C.; Brugnara,C. HPLC measurement, blood distribution, and pharmacokinetics of oral clotrimazole, potentially useful antisickling agent, *Clin.Chem.*, **1995**, *41*, 387–391.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 2 mL 95% EtOH and 2 mL MeCN:water 15:85. 100 μL Plasma + 50 μL 4 μg/mL + 3 mL MeCN:water 15:85, vortex for 30 s, add to the SPE cartridge, wash with 9 mL MeCN:water 40:60, dry the SPE cartridge, elute with 500 μL dichloromethane:MeOH 50:50. Evaporate the eluate to dryness, reconstitute the residue in 100 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil LC-1
Mobile phase: MeCN:MeOH:25 mM pH 6.3 K_2HPO_4 30:30:40
Flow rate: 2
Injection volume: 10
Detector: UV 263

CHROMATOGRAM
Retention time: 2.1
Internal standard: R051012 (Jannsen) (2.8)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, aspirin, barbituric acid, brompheniramine, caffeine, carbamazepine epoxide, carbamazepine, chloramphenicol, chlorpheniramine, clonazepam, clotrimazole, desipramine, desmethyldoxepin, digitoxin, digoxin, disopyramide, doxepin, ethosuximide, felbamate, gentamicin, ibuprofen, imipramine, lidocaine, maprotiline, mephenytoin, mephobarbital, metharbital, methsuximide, methylsuccinimide, nortriptyline, paramethadione, phenacemide, phenobarbital, phensuximide, phenylpropanolamine, phenytoin, primidone, protriptyline, theophylline, tobramycin, trimethadione, vancomycin

KEY WORDS
SPE; plasma

REFERENCE
Rifai,N.; Sakamoto,M.; Platt,O.; Brugnara,C. A high-performance liquid chromatographic assay for the determination of itraconazole concentration using solid-phase extraction and small sample volume, *Ther.Drug Monit.*, **1995**, *17*, 522–525.

SAMPLE
Matrix: blood, tissue
Sample preparation: Tissue. Homogenize 25 mg tissue with 1 mL MeOH. Add 50 μL 2.5 μg/mL IS in MeOH, vortex. Centrifuge the sample at 833 g for 15 min. Evaporate the supernatant to dryness under a stream of nitrogen. Reconstitute the residue in 300 μL MeOH, inject an aliquot. Plasma. Add 50 μL 12.5 μg/mL IS to 100 μL plasma, vortex. Add 300 μL MeOH, vortex for 1 min. Centrifuge the sample at 833 g for 5 min. Inject a 190 μL aliquot.

HPLC VARIABLES
Guard column: Novapak Guard-Pak

Column: 100 × 8 4 μm Novapak C18
Mobile phase: MeCN:diethylamine:water 58:0.05:42, adjusted to pH 2.45 with 85% phosphoric acid
Flow rate: 1.5
Injection volume: 190
Detector: F ex 260 em 365

CHROMATOGRAM
Retention time: 14.10
Internal standard: R51012 (Janssen Research Diagnostics, USA) (18.5)
Limit of detection: 5 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; serum; liver; bird

REFERENCE
Cox,S.K.; Orosz,S.; Burnette,J.; Frazier,D. Microassay for determination of itraconazole and hydroxyitraconazole in plasma and tissue biopsies, *J.Chromatogr.B*, **1997**, *702*, 175–180.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize (Ultra-Turrax) tissue in water 1:4. 1-2 mL Plasma or homogenate + 100 μL 2-10 μg/mL IS in MeOH + 50 mM pH 7.8 phosphate buffer, extract twice with 4 mL heptane:isoamyl alcohol 98.5:1.5 for 10 min. Combine the organic layers and add them to 3 mL 50 mM sulfuric acid, extract, centrifuge at 1000 g. Remove the aqueous phase and adjust the pH to 9 with concentrated ammonia, extract twice with 2 mL heptane:isoamyl alcohol 98.5:1.5. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 55°, reconstitute the residue in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.1 5 μm RSiL C18HL octadecyl (Alltech)
Mobile phase: MeCN:water 60:40 containing 0.05% diethylamine
Flow rate: 0.5
Injection volume: 40
Detector: UV 263

CHROMATOGRAM
Retention time: 4.3
Internal standard: cis-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-5-methyl-2-(3-methylbutyl)-3H-1,2,4-triazol-3-one (R 51 012) (5.8)
Limit of detection: 1 ng/mL

KEY WORDS
plasma; human; rat; pharmacokinetics

REFERENCE
Woestenborghs,R.; Lorreyne,W.; Heykants,J. Determination of itraconazole in plasma and animal tissues by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 332–337.

SAMPLE
Matrix: blood, tissue
Sample preparation: Tissue. Homogenize (Baxter Scientific disposable tissue grinder) esophageal tissue with 20 volumes of cold phosphate-buffered saline (pH 7.8). 250 μL Homogenate + 1 mL MeCN + 1 μL 1 mg/mL IS, vortex for 1 min, centrifuge at 1000 g for 5 min. Remove the supernatant and dry in air for 30 min, reconstitute in 100 μL mobile phase, inject an aliquot. Plasma. 250 μL Plasma + 1 mL MeCN + 1 μL 1 mg/mL IS, vortex for 1 min, centrifuge at

1000 g for 5 min. Remove the supernatant and dry in air for 30 min, reconstitute in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-pak C18
Mobile phase: MeCN:10 mM K_2HPO_4 60:40, final pH adjusted to 7.8 with 85% phosphoric acid
Flow rate: 1.3
Detector: UV 263

CHROMATOGRAM
Retention time: 4.35
Internal standard: R51012 (Research Diagnostics Inc.) (6.00) Remarks
Limit of detection: 10 ng/g, 5 ng/mL

OTHER SUBSTANCES
Noninterfering: ampicillin, cefazolin, cefoperazone, ceftriaxone, cefuroxime, clindamycin, fluconazole, folic acid, minocycline, nafcillin, norfloxacin, rifampin, tetracycline, vancomycin, zalcitabine, zidovudine

KEY WORDS
plasma

REFERENCE
Darouiche,R.O.; Setoodeh,A.; Anaissie,E.J. Potential use of a simplified method for determination of itraconazole levels in plasma and esophageal tissue by using high-performance liquid chromatography, *Antimicrob.Agents Chemother.*, **1995**, *39*, 757–759.

SAMPLE
Matrix: formulations
Sample preparation: 250 μL Syrup + 1.5 mL DMF, make up to 10 mL with mobile phase. 250 μL Solution + 500 μL 1 mg/mL IS, make up to 10 mL with mobile phase, inject a 5 μL aliquot.

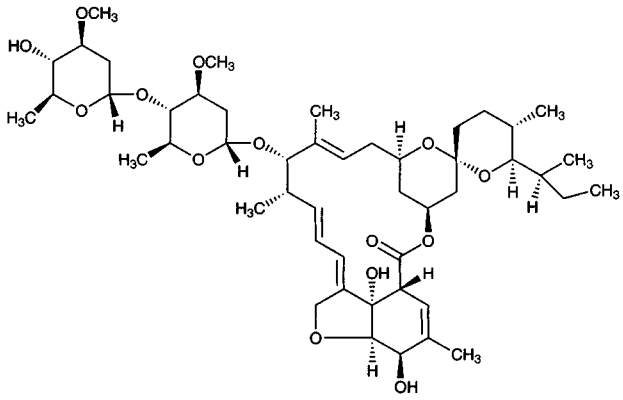
HPLC VARIABLES
Column: 250 × 4.6 5 μm Spheri-5 ODS
Mobile phase: MeCN:water:diethylamine 60:40:0.05
Flow rate: 1
Injection volume: 5
Detector: UV 263

CHROMATOGRAM
Retention time: 7
Internal standard: R51012 (Janssen) (11)

KEY WORDS
syrup; stability-indicating; suspensions

REFERENCE
Jacobson,P.A.; Johnson,C.E.; Walters,J.R. Stability of itraconazole in an extemporaneously compounded oral liquid, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 189–191.

Ivermectin



Molecular formula: $C_{48}H_{74}O_{14}$ (B1a)
Molecular weight: 875.11 (B1a)
CAS Registry No.: 70288-86-7, 70161-11-4 (B1a), 70209-81-3 (B1b)
Merck Index: 5264

SAMPLE

Matrix: blood

Sample preparation: Mix 5 mL Serum with 5.0 mL MeOH:buffer 20:80. Pass through an immunoaffinity column (80 × 7 mm, 1.0 mL bed volume, IgG + CNBr-activated Sepharose 4B, Pharmacia, Sweden) at 1.2 mL/min. Wash with 20 mL MeOH:buffer 10:90 and 5 mL MeOH:water 10:90. Elute with 5 mL MeOH. Evaporate the eluate to dryness at 55°. Add 1 mL MeOH to the residue, vortex for 15 s, inject an aliquot. (Buffer was prepared by dissolving 200 mg KH_2PO_4, 2.9 g $Na_2HPO_4.12\ H_2O$, 200 mg KCl, and 18.8 g NaCl in 900 mL water, adjusting to pH 7.4 with 2 M NaOH, and making up to 1 L with water.)

HPLC VARIABLES

Column: 220 × 4.6 5 μm Spheri-5 RP-18
Mobile phase: MeOH:water 95:5
Flow rate: 1.0
Injection volume: 10
Detector: UV 245

CHROMATOGRAM

Retention time: 6.5
Limit of detection: 200 pg/mL
Limit of quantitation: 2 ng/mL

KEY WORDS

serum; sheep; immunoaffinity; SPE

REFERENCE

Li,J.; Zhang,S. Immunoaffinity column cleanup and liquid chromatographic method for determining ivermectin in sheep serum, *J.AOAC Int.*, **1996**, *79*, 1300–1302.

SAMPLE

Matrix: blood

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 4 mL MeCN:water 50:50. Condition a Sep-Pak silica SPE cartridge with 4 mL MeCN and 4 mL dichloromethane. 5 mL Serum + 5 mL MeCN:water 50:50, add to the C18 SPE cartridge, wash with 4 mL MeCN:water 50:50, blow out excess solvent, elute the C18 SPE cartridge onto the silica SPE cartridge with 4 mL MeCN:dichloromethane 10:90, discard the C18 cartridge and elute the silica cartridge with 4 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 100 × 8 4 μm Nova-Pak radial PAK
Mobile phase: MeCN:MeOH:water 45:45:10
Flow rate: 1
Detector: UV 245

CHROMATOGRAM
Retention time: 4
Limit of detection: 2 ppb

KEY WORDS
cow; serum; SPE

REFERENCE
Oehler,D.D.; Miller,J.A. Liquid chromatographic determination of ivermectin in bovine serum, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 59–59.

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg Sep-Pak C18 SPE cartridge with 4 mL MeCN, 5 mL chloroform, 4 mL MeCN, and 4 mL water. 1 mL Plasma + 500 μL MeCN + 500 μL 1 ng/mL IS in MeCN, mix for 15 s, centrifuge at 2500 g for 10 min, add the supernatant to the SPE cartridge, dry under vacuum for 15 min, elute with 5 mL chloroform. Evaporate the eluate to dryness under a stream of nitrogen at <50°, reconstitute with 100 μL N-methylimidazole:MeCN 1:1, add 150 μL trifluoroacetic anhydride:MeCN 1:2, let stand for <30 s, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: MeCN:THF:water 40:40:20
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: F ex 365 em 475

CHROMATOGRAM
Retention time: 18 (ivermectin B_{1a})
Internal standard: avermectin B_{1a} (12.5)
Limit of detection: 20 pg/mL

KEY WORDS
plasma; SPE; cow; derivatization

REFERENCE
de Montigny,P.; Shim,J.S.K.; Pivnichny,J.V. Liquid chromatographic determination of ivermectin in animal plasma with trifluoroacetic anhydride and *N*-methylimidazole as the derivatization reagent, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 507–511.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL C18 SPE cartridge (J.T. Baker) with 4 mL MeCN, 5 mL chloroform, 4 mL MeCN, and 4 mL water. 1 mL Plasma + 50 μL 86-285 ng/mL IS in MeCN, vortex for 15 s, add 1 mL MeCN, mix, centrifuge at 1130 g for 15 min, add the supernatant to the SPE cartridge. Reconstitute the residue in 3.5 mL MeCN:water 1:2, vortex for 15 s, centrifuge at 1130 g for 15 min, add the supernatant to the SPE cartridge. Wash the SPE cartridge with 4 mL MeCN:water 1:2, dry under vacuum for 1 h, elute with 5 mL MeCN:chloroform 50:50. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute with two 100 μL portions of MeCN, evaporate to dryness under a stream of nitrogen at room temperature. Reconstitute with 100 μL N-methylimidazole:MeCN 1:1, add 150 μL trifluoroacetic anhydride:MeCN 1:2, let stand for 1.7 min, inject a 150 μL aliquot.

HPLC VARIABLES
Column: 100 × 2 3 μm MOS-Hypersil-2
Mobile phase: Gradient. MeCN:water from 72:28 to 92:8 over 15 min.
Flow rate: 0.3
Injection volume: 150
Detector: F ex 365 em 475

CHROMATOGRAM
Retention time: 22.5
Internal standard: ivermectin monosaccharide (20)
Limit of detection: 10 pg/mL

KEY WORDS
plasma; SPE; dog; narrow bore; derivatization

REFERENCE
Rabel,S.R.; Stobaugh,J.F.; Heinig,R.; Bostick,J.M. Improvements in detection sensitivity for the determination of ivermectin in plasma using chromatographic techniques and laser-induced fluorescence detection with automated derivatization, *J.Chromatogr.*, **1993**, *617*, 79–86.

SAMPLE
Matrix: blood, tissue
Sample preparation: Whole blood, serum. Condition a 1 mL Bond Elut C18 SPE cartridge with 2 mL MTBE, 2 mL MeCN, and 2 mL MeCN:water 50:50. 500 μL Whole blood or serum + 20 μL IS solution + 50 μL 200 mM zinc sulfate solution + 500 μL MeCN, vortex, centrifuge for 5 min, add to the SPE cartridge, wash with 2 mL MeCN:water 50:50, elute with 2 mL MTBE. Evaporate the eluate and dissolve the residue in 150 μL mobile phase, inject a 50 μL aliquot. Muscle. Condition a 3 mL Bond Elut C18 SPE cartridge with 4 mL MTBE, 4 mL MeCN, and 4 mL MeCN:water 50:50. Weigh out 1 g muscle, add 50 μL/g IS solution, add 6 mL MeCN:water 50:50, homogenize (Vertis 45), rinse blades and jar with 2 mL MeCN:water 50:50, add 200 mL (sic) 200 mM zinc sulfate, mix, centrifuge, add the supernatant to the SPE cartridge, wash with 4 mL MeCN:water 50:50, elute with 4 mL MTBE. Evaporate the eluate and dissolve the residue in 150 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 70 × 4.6 3 μm Ultrasphere XL ODS
Mobile phase: MeCN:MeOH:water 49:33:18
Column temperature: 56
Flow rate: 1
Injection volume: 50
Detector: UV 245

CHROMATOGRAM
Retention time: 5.6
Internal standard: dehydroivermectin (13.4) (Prepare by evaporating 1 mL 278 μg/mL ivermectin in MeCN into a tube. Add 200 μL 1-methylimidazole, add 300 μL acetic anhydride, add 900 μL DMF, mix well, heat at 60° for 15 min, add 4 mL MeCN, pass through a silica SPE cartridge, Evaporate the eluate to 500 μL, make up to 20 mL with MeCN, use this solution.)
Limit of detection: 2 ng/g (tissue), 2 ng/mL (whole blood, serum)

KEY WORDS
whole blood; serum; muscle; human; cow; SPE

REFERENCE
Dickinson,C.M. Improved high-performance liquid chromatographic method for quantitation of ivermectin in whole blood, serum or muscle tissue, *J.Chromatogr.*, **1990**, *528*, 250–257.

SAMPLE
Matrix: feces
Sample preparation: Stir 5 g feces with 25 mL MeOH for 25 min, centrifuge at 1500 g for 15 min, concentrate the supernatant to 7 mL under reduced pressure at 80°, centrifuge at 1500 g for 15 min, add procaine to a concentration of 50 ppm, make up to 10 mL with MeOH, filter (0.50 μm PTFE), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 300 × 9 Bondclone 10C18 (Phenomenex)
Mobile phase: MeCN:MeOH:water 47:33:20
Flow rate: 1

Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: 12.6
Internal standard: procaine (7.3)
Limit of detection: 20 ng/g

KEY WORDS
cow

REFERENCE
Bernal,J.L.; Del Nozal,M.J.; Salas,M.; Galante,E.; Lumaret,J.P. HPLC determination of ivermectin in cattle dung following subcutaneous injection, *J.Liq.Chromatogr.*, **1994**, *17*, 2429–2444.

SAMPLE
Matrix: milk
Sample preparation: Prepare a SPE cartridge by adding 2 g 40 μm Bondesil C18 18% load endcapped (Varian) to a 25 mL syringe barrel fitted with a 20 μm frit, wash with 5 mL petroleum ether, 5 mL acetone, and two 5 mL aliquots of MeOH, aspirate with full vacuum for <5 s (A). Condition a 500 mg Bond Elut LRC silica SPE cartridge with 3 mL hexane:ethyl acetate 60:40 (B). Condition a 500 mg Bond Elut LRC silica SPE cartridge with 4 mL chloroform (C). 25 mL Milk + 200 μL 500 ng/mL abamectin (avermectins) in MeOH, mix, add 5 mL to the SPE cartridge (A), mix milk with C18 material, let stand for 2 min, wash spatula with water, wash with two 5 mL portions of water, elute with 10 mL ethyl acetate, allow eluate to pass through a 5 cm layer of anhydrous sodium sulfate. Evaporate the eluate to dryness under a stream of nitrogen below 50°, add 2 mL hexane:ethyl acetate 60:40 to the oily residue, vortex, sonicate for 1 min, add mixture to SPE cartridge (B), rinse in with 1 mL hexane:ethyl acetate 60:40, wash with 5 mL hexane:ethyl acetate 60:40, elute with 5 mL MeOH:ethyl acetate 50:50. Evaporate the eluate to dryness under a stream of nitrogen below 60° (this residue should have no moisture in it), reconstitute the residue in 100 μL reagent, vortex gently for a few s, heat at 95° for 1 h, cool, add 1 mL chloroform, vortex, add to SPE cartridge (C), wash in with three 1 mL portions of chloroform, elute with 2 mL chloroform. Collect all the eluate and evaporate it to dryness under a stream of nitrogen below 60°, reconstitute in 500 μL MeOH, inject a 50 μL aliquot. (Prepare reagent by sequentially mixing 900 μL DMF, 300 μL acetic anhydride, and 200 μL N-methylimidazole just before use.)

HPLC VARIABLES
Guard column: Newguard RP-18 (Brownlee)
Column: 250 × 4.6 5 μm Econosil C18
Mobile phase: MeOH:THF:water 85:15:5
Flow rate: 1
Injection volume: 50
Detector: F ex 364 em 455

CHROMATOGRAM
Retention time: 15
Internal standard: abamectin (avermectins) (10.5)
Limit of detection: <1 ppb

KEY WORDS
cow; SPE; MSPD; derivatization; silylate glassware

REFERENCE
Schenck,F.J. Isolation and quantification of ivermectin in bovine milk by matrix solid phase dispersion (MSPD) extraction and liquid chromatographic determination, *J.Liq.Chromatogr.*, **1995**, *18*, 349–362.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Isolute C8 end-capped SPE cartridge (International Sorbent Technologies) with 5 mL MeCN and 5 mL MeCN:water 1:2 containing 0.1% triethylamine. Condition a 3 mL 500 mg Isolute silica SPE cartridge (International Sorbent Technologies) with 5 mL ethyl acetate:hexane 40:60. Homogenize (Tissuemizer) 5 g tissue in 25 mL MeCN, centrifuge at 3000 rpm for 5 min, decant supernatant into 50 mL water and 75

μL triethylamine. mix, pass through the C8 SPE cartridge at 2 mL/min, discard eluate, dry column under vacuum for 5 min, elute with 5 mL MeCN. Dry the eluate under a stream of nitrogen at 50-55°, resolubilize dry residues in 5 mL of ethyl acetate:hexane 40:60, vortex briefly, pass through the silica SPE cartridge at 2 mL/min. Rinse the reservoir with 5 mL ethyl acetate:hexane 40:60, add the rinse to the SPE cartridge. Dry the SPE cartridge for 5 min, elute with 5 mL MeOH:ethyl acetate 50:50, dry the eluate under a stream of nitrogen at 50-55°. Reconstitute the extract with 200 μL of fresh methylimidazole:MeCN 50:50, vortex briefly, add 300 μL of fresh trifluoroacetic anhydride:MeCN 1:2, vortex briefly, dry under a stream of nitrogen at 50-55° for 15 min. Add 500 μL MeOH:ammonium acetate:molecular sieves 4:1:1), vortex briefly, dry under a stream of nitrogen at 50-55°. Add 1 mL MeCN, vortex thoroughly, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil ODS (C18)
Mobile phase: MeCN-water 90:10
Column temperature: 65
Flow rate: 1.0
Injection volume: 50
Detector: F ex 272 em 465

CHROMATOGRAM
Retention time: 11 (B1b), 12.5 (B1a)
Limit of detection: 0.25 ppb

OTHER SUBSTANCES
Extracted: doramectin

KEY WORDS
SPE; derivatization; salmon; muscle

REFERENCE
Rupp,H.S.; Turnipseed,S.B.; Walker,C.C.; Roybal,J.E.; Long,A.R. Determination of ivermectin in salmon muscle tissue by liquid chromatography with fluorescence detection, *J.AOAC Int.*, **1998**, *81*, 549–553.

SAMPLE
Matrix: tissue
Sample preparation: Condition a C8 Bond-Elut SPE cartridge with 5 mL MeCN and 5 mL MeCN:water 30:70 containing 0.1% triethylamine. Mix 5 g muscle with 15 mL MeCN in a high speed blender for 1 min. Centrifuge at 3000 rpm for 5 min. Dilute 15 mL of the supernatant with 35 mL water, add 50 μL triethylamine, add to the SPE cartridge, elute with 5 mL MeCN, evaporate the eluate to dryness under a stream of nitrogen at 60°. Dissolve the residue in 1 mL MeOH, filter (0.45 μm Millipore filter). Transfer a 500 μL aliquot to a silanized 3 mL reaction vial, evaporate to dryness. Add 100 μL derivatization reagent, heat at 95° for 1 h. Cool to room temperature, add 1 mL chloroform, vortex, add quantitatively to a Sep-Pak silica SPE cartridge with 3-4 mL chloroform. Elute with 9 mL chloroform, evaporate the combined chloroform eluates to dryness. Dissolve the residue in 500 μL MeOH, filter (0.45 μm Millipore filter), inject a 50 μL aliquot. (Caution! Chloroform is a carcinogen! Derivatization reagent was 1-methylimidazole:acetic anhydride:DMF 2:6:9, freshly prepared.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Shandon ODS Hypersil C18
Mobile phase: MeOH:water 97:3
Column temperature: 30
Flow rate: 1
Injection volume: 50
Detector: F ex 364 em 470

CHROMATOGRAM
Retention time: 14
Limit of detection: 500-1000 ng/kg

KEY WORDS
pig; muscle; SPE; derivatization

REFERENCE
Nordlander,I.; Johnsson,H. Determination of ivermectin residues in swine tissues--an improved clean-up procedure using solid-phase extraction, *Food Addit.Contam.*, **1990**, *7*, 79–82.

SAMPLE
Matrix: tissue
Sample preparation: Add 15 mL MeOH to 5 g ' homogenized liver, shake thoroughly by hand and again using a shaking apparatus at medium speed for 1 h. Adjust the volume to 20 mL with MeOH, shake, centrifuge at 2000 g for 5 min. Mix 10 mL supernatant with 40 mL phosphate buffer A, add to immunoaffinity column (column preparation described in detail in paper) at 1.2 mL/min, wash with 40 mL MeOH:phosphate buffer B 10:90 and 10 mL MeOH:water 20:80. Elute with 3 mL MeOH, evaporate the eluate to less than 1 mL on a rotary evaporator at 55°, vortex with 5 mL ethyl acetate for 15 s. Collect the organic layer, evaporate to dryness at 55°, redissolve the residue in 1 mL mobile phase by vortexing for 15 s, filter (0.45 μm filter), inject a 100 μL aliquot of the filtrate. (Phosphate buffer A was 200 mg KH_2PO_4, 2900 mg Na_2HPO_4, 200 mg KCl, and 8800 mg NaCl in 900 mL water adjusted to pH 7.4 with 2 M NaOH and diluted to 1 L with water. Phosphate buffer B was 200 mg KH_2PO_4, 2900 mg Na_2HPO_4, 200 mg KCl, and 29.3 g NaCl in 900 mL water adjusted to pH 7.4 with 2 M NaOH and diluted to 1 L with water.)

HPLC VARIABLES
Column: 220 × 4.6 5 μm Brownlee C18
Mobile phase: MeCN:MeOH:water 45:45:10
Flow rate: 1
Injection volume: 100
Detector: UV 245

CHROMATOGRAM
Retention time: 15
Limit of detection: 2 mg/g
Limit of quantitation: 5 mg/g

KEY WORDS
immunoaffinity; liver; pig; SPE

REFERENCE
Li,J.S.; Li,X.W.; Hu,H.B. Immunoaffinity column cleanup procedure for analysis of ivermectin in swine liver, *J.Chromatogr.B*, **1997**, *696*, 166–171.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Bond-Elut C8 SPE cartridge (Analytichem International) with 5 mL MeCN and 5 mL MeCN:water 30:70 containing 0.1% triethylamine. Mix 5 g muscle with 15 mL MeCN in a high speed blender for 1 min, centrifuge at 3000 rpm for 5 min. Dilute 15 mL supernatant with 35 mL water, add 50 μL triethylamine, add the entire mixture to the SPE cartridge, elute with 5 mL MeCN, evaporate the eluate to dryness under a stream of nitrogen at 60°. Dissolve the residue in 1 mL MeOH and filter (0.45 μm). Evaporate a 500 μL aliquot of the filtrate to dryness in a silanized vial, add 100 μL derivatization reagent (1-methyl-imidazole:acetic anhydride:dimethylformamide 2:6:9, freshly prepared), heat in an oven at 95° for 1 h. Cool to room temperature, add 1 mL chloroform and vortex briefly. (Caution! Chloroform is a carcinogen!) Transfer quantitatively to a Sep-Pak silica SPE cartridge with 3-4 mL chloroform, elute with 9 mL chloroform, evaporate the combined chloroform eluates to dryness. Dissolve the residue in 500 μL MeOH, filter (0.45 μm), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil ODS C18 (Shandon)
Mobile phase: MeOH:water 97:3
Column temperature: 30
Flow rate: 1
Injection volume: 50
Detector: F ex 364 em 470

CHROMATOGRAM
Retention time: 14 (dihydroavermectin B1A)
Limit of quantitation: 500-1000 ng/g

KEY WORDS
derivatization; muscle; SPE; pig

REFERENCE
Nordlander,I.; Johnsson,H. Determination of ivermectin residues in swine tissues--an improved clean-up procedure using solid-phase extraction, *Food Addit.Contam.*, **1990**, *7*, 79–82.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeCN and 5 mL MeCN:water:triethylamine 30:70:0.1. Homogenize (Silverson) 5 g frozen minced tissue with 15 mL MeCN at full speed for 1 min, centrifuge at 4° at 2000 g for 10 min. Remove a 13 mL aliquot of the supernatant and add it to 35 mL water and 50 μL triethylamine, elute with 5 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 200 μL 1-methylimidazole:MeCN 1:1, add 300 μL trifluoroacetic anhydride:MeCN 1:2, mix, store cold, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 5 ODS-3
Mobile phase: MeOH:water 96:4
Flow rate: 1.8
Detector: F ex 364 em 470

CHROMATOGRAM
Retention time: 6 (ivermectin B_{1a})
Limit of detection: 1 ng/g

KEY WORDS
SPE; salmon; derivatization; brain; gill; kidney; liver; muscle; skin; spleen

REFERENCE
Kennedy,D.G.; Cannavan,A.; Hewitt,S.A.; Rice,D.A.; Blanchflower,W.J. Determination of ivermectin residues in the tissues of Atlantic salmon (*Salmo salar*) using HPLC with fluorescence detection, *Food Addit.Contam.*, **1993**, *10*, 579–584.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg Bond Elut C18 SPE cartridge with 5 mL MeCN and 5 mL MeCN:water:triethylamine 30:70:0.1. Homogenize (Polytron) 5 g tissue and 15 mL MeCN for 20 s, rinse probe with 5 mL MeCN, shake mechanically at high speed for 5 min, centrifuge at 2000 g for 5 min. Re-extract the solid with 10 mL MeCN. Add the supernatants to the alumina column. Combine the eluates, add 70 mL water, add 100 μL triethylamine, mix, add to the C18 SPE cartridge, pull air through the SPE cartridge for 3 min, elute with 5 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL freshly prepared reagent, vortex for 15 s, heat at 95-100° for 45 min, cool, add 1 mL chloroform, vortex, add to a 2.8 mL 500 mg Bond Elut silica SPE cartridge, elute with three 3 mL aliquots of chloroform. Combine the eluates and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL MeOH, filter, inject a 40 μL aliquot. (Prepare alumina column as follows. Shake 94 g Brockman Activity I neutral alumina (Fisher) and 6 mL water for 45 min, add 4.5 g alumina to an 8 mL column with a frit. Reagent was 200 μL 1-methylimidazole, 600 μL acetic anhydride, and 900 μL DMF.)

HPLC VARIABLES
Guard column: 30 × 4.6 RP-18 (Brownlee)
Column: 250 × 4.6 RP-18 OD-224 (Brownlee)
Mobile phase: MeOH:water 97:3
Flow rate: 1.8

Injection volume: 40
Detector: F ex 365 em 425

CHROMATOGRAM
Retention time: 9.3
Limit of detection: 2 ppb

KEY WORDS
SPE; cow; pig; sheep; fish; liver; muscle; derivatization

REFERENCE
Salisbury,C.D.C. Modified method for the determination of ivermectin residues in animal tissues, *J.AOAC Int.*, **1993**, *76*, 1149–1151.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 4 mL MeCN and 4 mL MeCN:water 10:90. 4 g Minced meat or liver + 40 mL MeCN + 3.5 mL water, vortex for 2 min, centrifuge at 2000 rpm for 10 min, remove supernatant, repeat extraction with 20 mL MeCN and 3.5 mL water. Combine the supernatants and evaporate them to 6 mL under reduced pressure (all the MeCN should be removed), add 6 mL water to the residue, add to the SPE cartridge, pull air through the cartridge for 10 min, elute with 5 mL MeCN. Evaporate the eluate under a stream of nitrogen, add 150 μL trifluoroacetic anhydride:MeCN 1:2 to the residue, add 100 μL N-methylimidazole:MeCN 50:50, shake, store in the dark, inject a 10-50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water 95:5 (At the end of the day flush column with 30 mL MeOH.)
Flow rate: 1.5
Injection volume: 10-50
Detector: F ex 364 em 470

CHROMATOGRAM
Retention time: 11
Limit of quantitation: 5 ng/g

KEY WORDS
meat; liver; cow; pig; muscle; SPE; derivatization

REFERENCE
Degroodt,J.M.; Wyhowski de Bukanski,B.; Srebrnik,S. Determination of ivermectin residues in meat and liver by HPLC and fluorometric detection, *J.Liq.Chromatogr.*, **1994**, *17*, 1419–1426.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL Bakerbond C8 SPE cartridge with 5 mL MeCN and 5 mL MeCN:water:triethylamine 30:70:0.1. Prepare a 55 mm column of 70-230 mesh Kiselgel 60 (Merck) in a Pasteur pipette, condition with 3 mL hexane:isopropanol 60:40. Homogenize (Polytron) 5 g blended tissue with 12 mL MeCN, rinse homogenizer with 3 mL MeCN, centrifuge the mixture at 4000 rpm for 10 min. Remove the supernatant and make up to 50 mL with water, add 50 μL triethylamine, shake, add to the SPE cartridge, elute with 5 mL MeCN at 1 drop/s. Evaporate the eluate to 300 μL under a stream of nitrogen at 40°, transfer to a smaller vial with MeCN, evaporate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL 1-methylimidazole:MeCN 50:50, cool in an ice bath, add 150 μL trifluoroacetic anhydride:MeCN 1:2, shake at room temperature for 1 min, add to the column, rinse the vial with 500 μL hexane:isopropanol 60:40, add the rinse to the column, elute with 1 mL hexane:isopropanol 60:40. Evaporate the eluate to dryness under a stream of nitrogen with heating, reconstitute with 250 μL MeCN, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm LiChrospher 100 RP-18

Column: 125 × 4 5 μm LiChrospher 60 RP-select B
Mobile phase: MeOH:water 95:5
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 365 em 465

CHROMATOGRAM
Retention time: 3.5
Limit of detection: 1 ppb
Limit of quantitation: 2.5 ppb

KEY WORDS
derivatization; muscle; liver; pig; cow; SPE

REFERENCE
Guggisberg,D.; Sievi,M.; Koch,H. Methode zur quantitativen Bestimmung von Ivermectin in Fleisch und Leber mit HPLC und Vorsäulenderivatisation [Method for the quantitative determination of ivermectin in meat and liver by HPLC and pre-column derivatization], *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene*, **1994**, *85*, 395–405.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg Bakerbond C18 SPE cartridge with three 5 mL portions of MeOH, 5 mL MeCN, and three 5 mL portions of MeCN:water:triethylamine 30:70:0.1. Condition a Waters silica SPE cartridge with 8 mL chloroform. Homogenize (Ultraturrax) 5 g minced tissue with 15 mL MeCN at high speed for 3 min, rinse blade with 2 mL MeCN, sonicate for 15 min, centrifuge at 3000 rpm for 5 min, filter (paper), extract the residue again with 10 mL MeCN, wash the filter with 3 mL MeCN. Combine the organic layers and add 70 mL water and 100 μL triethylamine, stir thoroughly, add to the C18 SPE cartridge, wash with two 5 mL portions of MeCN:water 50:50, elute with 7 mL MTBE at 2 mL/min. Store the eluate overnight at -20°, remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 3 mL MeOH, add 100 μL water, add 3 mL hexane, vortex, remove the hexane layer, repeat the hexane wash. Extract the combined hexane layers with 1 mL MeOH. Combine the MeOH layers and evaporate them to dryness under a stream of nitrogen at 50°, heat in a vacuum oven at 50° for 30 min, reconstitute the residue in 150 μL 1-methylimidazole:acetic anhydride:DMF 2:3:9 (freshly prepared), vortex for 30 s, heat at 100° for 1 h, cool, add 1 mL chloroform, vortex, add to the silica SPE cartridge, elute with three 3 mL portions of chloroform. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 400 μL MeOH, vortex, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil LC-18
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:water 95:5
Flow rate: 1.8
Injection volume: 20
Detector: F ex 360 em 470

CHROMATOGRAM
Retention time: 7
Limit of detection: 2 ng/g

KEY WORDS
derivatization; SPE; liver; muscle; fat; guinea pig; cow; pig; horse; sheep; pharmacokinetics

REFERENCE
Dusi,G.; Curatolo,M.; Fierro,A.; Faggionato,E. Determination of the antiparasitic drug ivermectin in liver, muscle and fat tissue samples from swine, cattle, horses and sheep using HPLC with fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1607–1616.